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Abstract

Fungi play a crucial role in dead wood decay, being the major decomposers of wood and affecting other members of the dead wood-associated microbiota. We sampled dead wood from five deciduous tree species over more than forty years of decay in a natural European floodplain forest with high tree species diversity. While the assembly of dead wood fungal communities shows a high level of stochasticity, it also indicates clear successional patterns, with fungal taxa either specific for early or late stages of wood decay. No clear patterns of fungal biomass content over time were observed. Out of 220 major fungal operational

taxonomic units, less than 8% were associated with single tree species, most of them with *Quercus robur*. Tree species and wood chemistry, particularly pH, were the most important drivers of fungal community composition. This study highlights the importance of dead wood and tree species diversity for preserving the biodiversity of fungi.

Keywords

Dead wood, Decomposition, Fungal community, Alluvial forest, Succession, Tree species diversity, Natural forest, Fungal biomass

1. Introduction

Dead wood is a fundamental element that contributes to the functioning of forest ecosystems. It represents a source of nutrients and a habitat for many organisms (Lassauce et al. 2011, Stokland et al. 2012, Baldrian 2017), as well as a major stock of recalcitrant carbon (C), especially in natural forests, i.e., not managed for timber production. The different tree species, sizes or decay stages that characterize dead wood create a diversity of microenvironments that host a large diversity of organisms (Rayner and Boddy 1988, Larrieu et al. 2014, Vítková et al. 2018), including endangered species (Hekkala et al. 2016, Müller et al. 2020, Nordén et al. 2020). Dead wood also constitutes a globally important stock of carbon (Pan et al. 2011) that contributes to soil formation and nutrient cycling at the ecosystem level (Šamonil et al. 2020, Tláskal et al. 2021). The carbon storage capacity of dead wood depends on environmental conditions, such as temperature and humidity (Błońska et al. 2019, Moreno-Fernández et al. 2020), as well as the tree species composition (Błońska et al. 2017). The more dead wood there is, the greater these ecosystem services will be ensured. Dead wood management thus may contribute to the mitigation of climate change (De Meo et al. 2019). In

this context, in managed Central European forests, it is currently considered beneficial to artificially create dead wood (e.g., tree girdling) (Vítková et al. 2018).

Natural forests, i.e., unmanaged forests, contain a higher amount of dead wood, including senescent trees, than managed forests (Siitonen et al. 2000, Luyssaert et al. 2008, Baldrian 2017, Vítková et al. 2018). In addition, tree density is often high, promoting competition among trees and leading to high tree mortality (Moreno-Fernández et al. 2020). Another important feature of natural forests is high tree species diversity (Paillet et al. 2010). Tree species diversity is positively correlated with forest productivity and dead wood volume (Doerfler et al. 2017, Zeller and Pretzsch 2019). Moreover, different tree species lead to different sizes and shapes of dead wood: spruce tends to uproot and forms large pieces of lying deadwood, pine tends to produce standing snags and birch mainly forms snags that crumble into small pieces (Šenhofa et al. 2020). Finally, each tree species has distinct physical properties and chemical compositions of dead wood. This implies varying rates of decomposition (Vrška et al. 2015, Přívětivý et al. 2017) and variation in metabolome composition that together result in largely heterogeneous environmental conditions for wood-associated organisms, favouring diversity (Přívětivý et al. 2017, Baldrian et al. 2021).

The decomposition of dead wood is a process induced by the combined action of multiple groups of organisms (Tláskal et al. 2021) and environmental conditions (Jacobs and Work 2012, Přívětivý et al. 2016). Invertebrates (Ulyshen 2016, Griffiths et al. 2019) and bacteria (Tláskal et al. 2017, Odriozola et al. 2020, Tláskal et al. 2021) have roles in decomposition, but the primary agents are fungi (Tláskal et al. 2021). Dead wood is mainly composed of cellulose, hemicellulose and lignin (Rayner and Boddy 1988). Different categories of fungi, mainly brown-rot, white-rot and soft-rot fungi, will develop into dead wood according to their ability to degrade these wood components (Goodell et al. 2008). Due to priority effects (Boddy 2000, Hiscox et al. 2015), the colonizers of fresh wood will

determine the early microbial communities in dead wood. Fresh wood has limited physical permeability, high lignin and low nitrogen (N) concentrations, but fungi are able to colonize this habitat. Indeed, fresh wood has a good longitudinal access, i.e., along vascular tissues, and endophytic fungi are latently present in wood while it is still functional in the standing tree (Boddy et al. 2016). Thus, the initial stochastic community inherited from fresh wood evolves into further decay stages, and the first colonizers influence the establishment of subsequent species (Rayner and Boddy 1988, Hiscox et al. 2015, Song et al. 2017). The decomposition of dead wood is accompanied by a reduction in wood density, an increase in water content, lignin and N content and a decrease in pH, although these patterns are not universal (Fukasawa et al. 2009, Baldrian et al. 2016, Rinne et al. 2017).

Among the drivers of the composition of the fungal community in dead wood, tree species have been designated as the most important factor regardless of the decay stage (Hoppe et al. 2016, Krah et al. 2018, Purahong et al. 2018c). One of the reasons for this is the size and physico-chemical properties of the dead wood of different tree species (Arnstadt et al. 2016, Baldrian et al. 2016, Kahl et al. 2017). The diversity of tree species has been identified as a major factor promoting the diversity of wood-inhabiting fungi (Krah et al. 2018), and the amount of dead wood is also important (Rondeux and Sanchez 2010, Blaser et al. 2013). The environmental conditions, such as moisture, can also affect the fungal community composition. Indeed, moisture changes weaken the wood coatings which increases wood physical permeability allowing fungal propagules to colonize dead wood (Goodell et al. 2020). Finally, decay stage is the last characteristic of dead wood that affects the structure and composition of the fungal community (Baldrian et al. 2016, Błońska et al. 2017). The interactions between all the abovementioned factors, i.e., tree species, decay stage and environment, determine the composition and structure of the fungal community in dead wood (Zuo et al. 2014).

In this study, we aimed to identify the link between tree species, decay stage and fungal community composition in a natural European floodplain forest containing high tree species diversity. Our aims were to: (i) detect the proportion of generalist vs specialist fungal species among all tree species, (ii) identify the drivers of fungal community structure and composition among tree species, decay stage or wood properties, and (iii) determine the importance of tree species in the succession of fungi and chemical properties of dead wood during the decay process. We hypothesized that high tree diversity may result in a significant proportion of tree-species specialist fungal taxa (Juutilainen et al. 2011, Krah et al. 2018). Among the drivers of fungal community structure and composition, we expected that tree species would dominate (Purahong et al. 2018c), but the effect of wood moisture would also be significant in this floodplain forest (Vrška et al. 2015). We hypothesized that the rate of successional change in fungal community composition over time would be higher in dead wood of tree species undergoing fast decomposition (Song et al. 2017). Since the colonizers of living wood (such as the colonizers of tree leaves) are often tree species-specific, we hypothesized that the proportion of specialist fungi would decrease over decomposition process, as shown for the decomposition of plant leaf litter (Štursová et al. 2020a).

2. Materials and methods

2.1. Study site and dead wood characteristics

The study was conducted in the Ranšpurk National Nature Reserve (48°40'43"N 16°56'56"E) in the Czech Republic. The Reserve covers an area of 22.25 ha and has an average elevation of 154 m a. s. l. The mean annual temperature is 9.9°C, and the mean annual precipitation is 545 mm (Šamonil et al. 2017). The majority of vegetation consists of a primeval alluvial forest preserved from any forestry activity since 1932. Before this date, the only activity was grazing. This natural temperate floodplain forest was selected because it contains high tree diversity (Rejšek 2007). The dominant tree species are *Acer campestre*,

126 *Fraxinus angustifolia*, *Carpinus betulus*, *Ulmus laevis* and *Quercus robur*. The absence of
127 any type of timber management or harvesting since the middle of the 20th century has resulted
128 in a large stock of dead wood. A census performed in 1994 recorded a total of 148 m^3 ha^{-1} of
129 dead wood and 563 m^3 ha^{-1} of living trees (Vrška et al. 2006). A detailed census of the
130 position and status, i.e., living or dead, of all trees with a diameter at breast height (DBH) ≥
131 10 cm was first conducted in 1973 (Šamonil et al. 2017) and repeated in 1994, 2006 and 2016.
132
133 *2.2. Dead wood sampling*
134 The sampling of dead wood was performed on October 3-5, 2016. For the present
135 study, we preselected coarse woody debris (CWD, i.e., tree trunks) belonging to five
136 dominant tree species, namely, *Acer campestre*, *Carpinus betulus*, *Fraxinus angustifolia*,
137 *Quercus robur* and *Ulmus laevis*, with an initial DBH between 27 and 177 cm at the time
138 when the tree was first recorded to have fallen (Supplementary data 1). This selection
139 comprised the bulk of the dead wood volume in the ecosystem. CWD with a DBH <27 cm
140 that rapidly decomposes and CWD with a DBH >177 cm that could not be representatively
141 sampled were excluded. Moreover, trees that decayed while standing before they fell were
142 also excluded because the length of time over which decomposition had occurred was unclear.
143 The selected CWD came from four decay classes defined by the year when the trees were first
144 recorded as fallen. Within each decay class, trees were randomly selected considering the
145 equal representation of DBH between 27 and 177 cm to obtain samples from 127
146 decomposing trees in total. The dead wood age classes were designated as follows: 1 (fallen
147 after 2006, <10 years of decomposition), 2 (fallen between 1994 and 2006, 10-21 years), 3
148 (fallen between 1973 and 1994, 22-43 years), and 4 (fallen before 1973, >44 years of
149 decomposition). The distance between logs typically ranged in the tens of metres. All

combinations of trees and decay lengths were sampled except for *Carpinus betulus* stage 4 since the CWD of this combination was not available.

Four CWD samples were obtained from each selected log using an electric drill with a bit diameter of 10 mm. The length of each CWD (or the sum of the lengths of its fragments) was measured, and samples were collected at 1/5, 2/5, 3/5 and 4/5 of the CWD length. Before drilling, mosses, lichens and bark were carefully removed. Drilling was performed vertically from the middle of the upper surface to a depth of 40 cm. The drill bit was properly cleaned with ethanol-soaked tissue to wipe off all visible particles between drillings, and sawdust was collected with gloves, in batches of two adjacent drill holes, and directly deposited in plastic bags and frozen at -20°C within a few hours after drilling.

2.3. Dead wood processing

In the laboratory, the sawdust material was processed as described previously (Baldrian et al. 2016). It was weighed, freeze-dried and milled with an Ultra Centrifugal Mill ZM 200 (Retsch, Germany). The parts of the centrifugal mill in contact with sawdust were carefully washed with a cleanser containing 4.7% of sodium hypochlorite, cleaned with ethanol and dried between each sample. The fine sawdust obtained after milling was used for the subsequent analyses. The water content was calculated as the difference between the wet mass and dry mass after freeze-drying, and expresses in g water per g wood dry mass. pH was measured in distilled water (1:10). The carbon (C) and nitrogen (N) contents were measured by an external laboratory (Research Institute for Soil and Water Conservation, Prague, Czech Republic) as described previously (Větrovský and Baldrian 2015). C was measured using sulfochromic oxidation (ISO 14235), and N was estimated by sulfuric acid mineralization with the addition of selenium and sodium sulphate and conversion to ammonium ions (ISO 11261), which were measured by a segmented flow analyser. The total ergosterol content,

which is an approximation of the fungal biomass, was extracted from 0.3 g of sawdust material using 10% KOH in methanol and analysed by high-performance liquid chromatography (Šnajdr et al. 2008).

From each of the two batches of sawdust initially collected from each CWD sample, total DNA was extracted from 200 mg of freeze-dried and milled sawdust material with the NucleoSpin Soil kit (Macherey-Nagel, Germany) following the manufacturer's instructions with the use of SL1 lysis buffer and SX enhancer. Thus, two extractions were performed for each CWD sample, and the two DNA extracts were pooled. The pools of DNA extract were quantified with a Qubit™ dsDNA BR Assay kit (Thermo Fisher Scientific) on a Qubit 2.0 fluorometer (Life Technologies). Then, they were diluted at 5 ng μl^{-1} in ddH2O, and 1 µl of this dilution was used for one 25 µl PCR reaction. Three PCRs per sample were performed to amplify the ITS2 region of fungal rDNA with the barcoded primers gITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990). The PCR mixture contained 5 ng of DNA in a 25 µl final volume containing 5 µl of Q5 Reaction Buffer (New England Biolabs, Inc.), 0.5 µl of 10 mM PCR Nucleotide Mix (Bioline), 1.5 µl of bovine serum albumin at 10 $mg.ml^{-1}$ (GeneON), 0.25 µl of Q5 High-Fidelity DNA polymerase (New England Biolabs, Inc.), 5 µl of Q5 High GC Enhancer (New England Biolabs, Inc.), 1 µl of 10 µM of each primer (Sigma-Aldrich) and 9.75 µl of H_2O. The PCR amplification conditions were 94°C for 5 min, 30 cycles of 30 s at 94°C, 56°C for 30 s and 72°C for 30 s, followed by 7 min at 72°C. The amplification was checked for each PCR product separately by loading them on 1% (g/ml) agarose gel electrophoresis (Agarose RA, VWR Life Science) in tris-acetate EDTA buffer 1X (Thermo Fisher Scientific). To visualize the PCR products, the agarose gel was stained with 1 mg/ml of ethidium bromide (Sigma-Aldrich). The gel was run using horizontal electrophoresis at 90V for 45 min. O'Gene Ruler 100 bp Plus DNA ladder (Thermo Fisher Scientific) was used as a marker for size determination of the PCR products. All the PCR products showed

amplification. The three PCR products were then pooled, purified using a MinElute kit (Qiagen) and quantified with a Qubit™ dsDNA BR Assay kit (Thermo Fisher Scientific) on a Qubit 2.0 fluorometer (Life Technologies). An amplicon library was prepared from the purified PCR products using the TruSeq DNA PCR-free kit (Illumina), and the resulting library was sequenced in-house with a MiSeq Reagent Kits v2 (Illumina) on an Illumina MiSeq platform (2×250 base paired-end reads). The sequence data have been deposited into the National Center for Biotechnology Information database under the accession number **PRJNA681341**.

2.4. Bioinformatic processing of sequencing data

The amplicon sequencing data were processed using the SEED 2 pipeline (Větrovský et al. 2018). We used only the forward reads for the analyses, as joining the paired-end reads would exclude fungal taxa with ITS2 region lengths higher than 400 bases, including abundant wood-decomposing fungi from the genus *Armillaria* (Větrovský et al. 2020). After quality filtering, the sequences with <40 bases were removed. The ITS2 region, even incomplete, was extracted using the ITSx software (Bengtsson-Palme et al. 2013) before processing. Then, chimeric sequences were identified and deleted using Usearch 8.1.1861, and the remaining sequences were clustered into operational taxonomic units (OTUs) using UPARSE implemented within Usearch (Edgar 2013) with a 97% similarity level. The global singletons, i.e., the OTUs represented by only one sequence across the 127 samples, were ignored. The most abundant sequence from each cluster was selected as the representative sequence used for cluster identification. The closest hits at the species level were identified using BLASTn against the UNITE 7.1 database (Nilsson et al. 2019). The nonfungal hits and sequences without identification were removed. The OTUs having the same species identification and, at the same time, a similarity ≥ 97% with a coverage ≥ 95% were merged

into a single taxon, and species-level identification was used. For the OTUs with lower similarity, lower coverage or both, genus-level identification, or the best available identification, was used. The OTUs with the same identification (e.g., "Fungi sp.") were numbered to differentiate them. Based on the published literature, the fungal genera of the best hits were used to assign putative ecophysiological categories (Põlme et al. 2020). The relative abundance data reported in this paper are based on the dataset of sequence relative abundances and should be taken as proxies of taxon abundances only with caution (Lindahl et al. 2013, Větrovský et al. 2016, Palmer et al. 2018). A parallel analysis was also performed on the OTU presence-absence table using the Jaccard distance (Shi 1993) (Supplementary data 2-4).

2.5. Statistical analyses

For the diversity analyses, the number of sequences for each sample was randomly subsampled at the same sequencing depth of 10,000 sequences. Eight samples having a sequencing depth below this threshold were not considered for diversity analyses. For the analyses of community composition and similarity, the same dataset was used, but the eight samples with a sequencing depth below 10,000 were included with all their sequences.

The statistical analyses were performed using R version 3.5.3 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). The effect of age class on wood properties (i.e., water, N, C, C/N, pH and ergosterol) was assessed for each tree species independently with one-way ANOVA and Tukey-Kramer HSD tests. Diversity indices of fungal communities were calculated with the 'vegan' R package, and the effects of age class, tree species and their interaction were evaluated with two-way ANOVAs. The structure of the fungal communities was visualized with two-dimensional nonmetric multidimensional scaling (NMDS) ordination analysis based on Bray-Curtis distances of OTU relative abundances.

NMDS was performed on the entire dataset and for each tree species independently with the 'metaMDS' function from the 'vegan' R package (Oksanen et al. 2016). The environmental variables (age class, water, N, C, C/N and pH) were fitted as vectors in the NMDS analyses using the 'envfit' function of the 'vegan' R package (Oksanen et al. 2016). PERMANOVA was used to determine the existence of differences in fungal community structure according to the tree species and age class. The 'betadisper' function of the 'vegan' R package (Oksanen et al. 2016) was used to test for homogeneity of multivariate dispersion. Variation partitioning analyses on Hellinger-transformed OTU abundances were performed to identify the part of the variance explained by age class, tree species or wood chemistry (i.e., water, N, C, C/N and pH) for the entire dataset and with age class and wood chemistry for each tree species independently. The 'varpart' function from the 'vegan' R package was used (Oksanen et al. 2016). The importance of the obtained variances was determined with Monte Carlo permutation tests. To determine the level of similarity in the fungal community composition during dead wood decomposition, Mantel tests were used for each tree species. They allowed us to examine the correlations between the OTU abundance matrix (i.e., Bray-Curtis dissimilarities of OTU relative abundances) and time matrix (i.e., Euclidean distance of time, in number of years, calculated from age classes) (Franklin and Mills 2009). The 'mantel' function of the 'vegan' R package was used (Oksanen et al. 2016).

For the detailed analysis of genus composition, only fungal genera represented by at least 5% relative abundance in one of the treatments (i.e., in one combination of tree species and age class) were used (32 genera in total), while for analyses of fungal specificity, the OTUs represented by at least 0.5% for at least three individual CWD samples were used (220 OTUs in total). To determine the level of specialization of these 220 OTUs over time for each tree species, we calculated the specificity, i.e., the number of tree hosts for each OTU and the mean succession stage. OTUs were assigned to all tree species where they were recorded with

a relative abundance of at least >0.1% in at least two samples or with a relative abundance >0.5% in at least one sample. The mean succession stage was defined as the mean position of the OTU in succession considering its relative abundances over time as described previously (Šťursová et al. 2020b). To study the variation in the number of tree hosts of fungi across their mean succession stage, first- second- and third-degree polynomial functions were used, and we tested the significance of these polynomial functions with the 'lm' function from R. Samples of *Carpinus betulus* were excluded since the CWD at stage 4 were not recorded. In all statistical analyses, the results with P-values < 0.05 were considered to be significant.

3. Results

3.1. Dead wood chemistry and fungal biomass

Dead wood chemistry was specific for each tree species independent of age class. The pH was globally lower in *Quercus robur* (pH=4.0±0.1, mean±standard error) than in *Acer campestre* (pH=5.7±0.3) ($P < 0.001$). The water content was also different among species, and the averages ranged from 52.0±3.0 (in *Quercus robur*) to 66.5±4.0 (in *Ulmus laevis*). Among the five tree species selected, *Fraxinus angustifolia* and *Ulmus laevis* were the only species for which dead wood chemistry differed among decay stages (Figure 1). In *Fraxinus angustifolia*, the water and N contents were lower at stage 1 and increased with time ($P < 0.001$ and $P = 0.026$, respectively). The pH significantly decreased with time in *Fraxinus angustifolia* and *Ulmus laevis* ($P < 0.001$ and $P = 0.015$, respectively).

The ergosterol content, which reflects fungal biomass, did not show clear trends with dead wood age for most tree species, but in *Carpinus betulus* and *Fraxinus angustifolia*, the ergosterol content was significantly lower at age class 1 than in older dead wood ($P = 0.004$ and $P < 0.001$, respectively). Typically, the highest content of fungal biomass was recorded at age classes 2 or 3 (Figure 1).

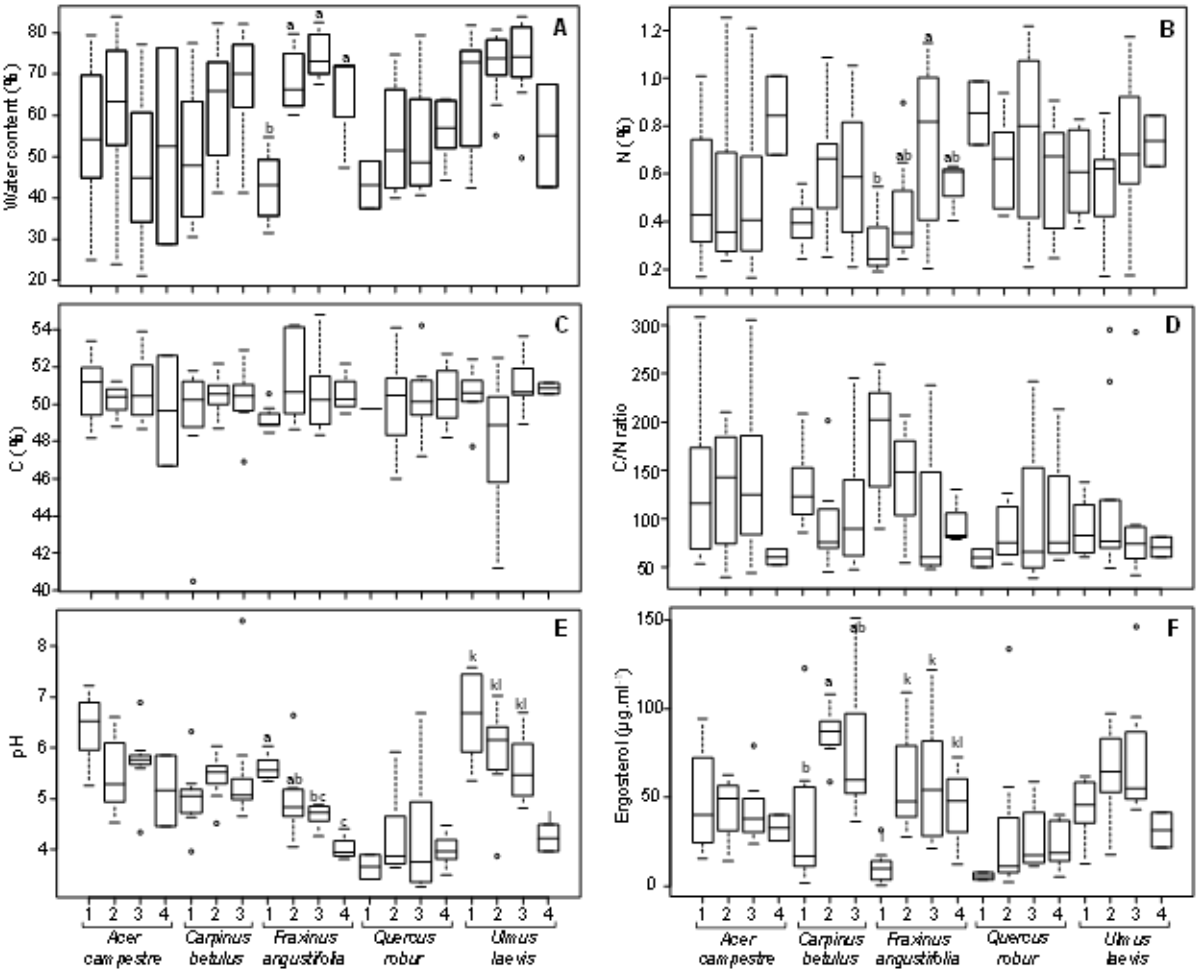

Fig. 1. Properties of decomposing dead wood in the natural floodplain forest. Bar plots represent medians, upper and lower quartiles and ranges of observed values. Different letters indicate significant differences between age classes after the Tukey-Kramer HSD test was performed for each tree species independently ($P < 0.05$).

3.2. Fungal community composition in dead wood

A total of 3,677,662 reads were obtained from the 127 CWD samples with on average 28,958 reads per sample (Supplementary data 1). In total, 81,509 OTUs were obtained and 21,809 OTUs were kept for further analyses after singletons removal. The fungal community was highly diverse globally (Supplementary data 5). The Shannon diversity index was tree-species specific ($F_{4,100}$=3.38; P = 0.012) and was significantly higher in *Quercus robur* (3.59±0.13) than in *Carpinus betulus* and *Ulmus laevis* (2.61±0.25 and 2.78±0.14, respectively). The lowest and highest values were both observed in age class 1, with

2.11±0.33 in *Carpinus betulus* and 3.99±0.11 in *Quercus robur*. OTU richness ranged from 351±33 (for *Ulmus laevis* – age class 3) to 759±380 (for *Fraxinus angustifolia* – age class 4), and the estimated richness reflected by the Chao1 index ranged from 713±221 (for *Ulmus laevis* – age class 4) to 1,776±806 (for *Fraxinus angustifolia* – age class 4) (Supplementary data 5). No significant differences in diversity with age class were observed.

The composition of fungal communities in dead wood was significantly affected by tree species ($P < 0.001$), age class ($P < 0.001$) and their interaction ($P = 0.004$) (Table 1). Since tree species was the most important predictor, the effect of age class was also explored for each tree species separately (Table 1). In this analysis, the age class effect was significant for *Fraxinus angustifolia* ($P = 0.002$) and *Quercus robur* ($P = 0.032$) and marginally significant for *Acer campestre* ($P = 0.066$). The NMDS analysis of the entire dataset showed separation of samples by age classes (Figure 2): class 4 was separated from the others along the second axis, and class 1 tended to be separated along the first axis. Concerning tree species, *Quercus robur* and *Fraxinus angustifolia* appeared to be most distinct (Figure 2). The NMDS analyses performed for individual tree species highlighted the difference in fungal community composition between classes 1 and 4 in *Quercus robur* and *Fraxinus angustifolia* (Supplementary data 6).

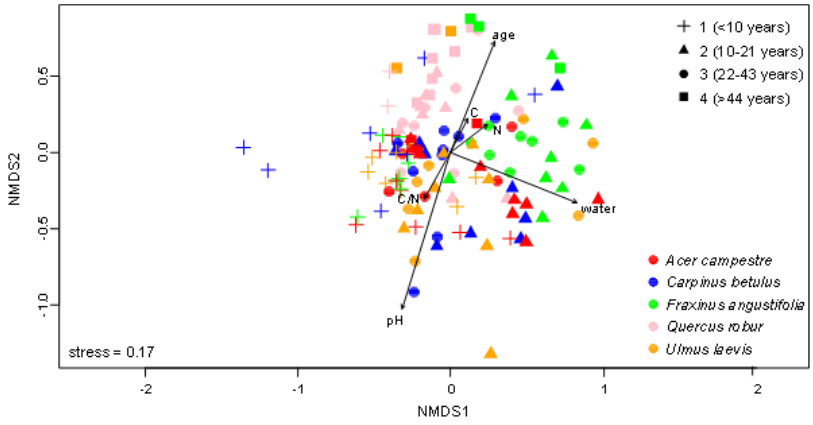

Fig. 2. Nonmetric multidimensional scaling (NMDS) of fungal communities in the dead wood samples from the natural floodplain forest. The NMDS analysis is based on the Bray-Curtis dissimilarities among the 127 samples. Vectors indicate environmental variables showing a significant effect.

	Whole model					*Acer campestre*					*Carpinus betulus*					*Fraxinus angustifolia*					*Quercus robur*					*Ulmus laevis*				
Adonis F-statistics from PERMANOVA tests																														
	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p
Age class	1	1.18	0.02	2.82	**0.001**	1	0.64	0.06	1.56	0.066	1	0.52	0.05	1.20	0.155	1	0.75	0.07	1.88	**0.002**	1	0.60	0.06	1.45	**0.032**	1	0.53	0.05	1.18	0.139
Tree species	4	4.01	0.07	2.39	**0.001**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Age class x Tree species	4	2.19	0.04	1.31	**0.004**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Residual	117	49.06	0.87			24	9.83	0.94			23	9.89	0.95			24	9.59	0.93			24	9.96	0.94			22	9.78	0.95		
Total	126	56.44	1.00			25	10.47	1.00			24	10.41	1.00			25	10.34	1.00			25	10.56	1.00			23	10.31	1.00		
Homogeneity of dispersion between PERMANOVA groups																														
	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p
Groups	18	0.18	0.010	2.36	**0.003**	3	0.04	0.013	1.51	0.241	2	0.01	0.003	1.15	0.336	3	0.02	0.007	1.85	0.168	3	0.03	0.011	2.86	0.060	3	0.04	0.013	4.96	**0.010**
Residuals	108	0.46	0.004			22	0.18	0.008			22	0.05	0.002			22	0.09	0.004			22	0.09	0.004			20	0.05	0.003		
Correlations between wood properties and the NMDS analyses																														
	r2	p				r2	p				r2	p				r2	p				r2	p				r2	p			
age	0.28	**0.001**				0.34	**0.006**				0.13	0.230				0.46	**0.002**				0.29	**0.015**				0.22	0.062			
water	0.36	**0.001**				0.25	**0.037**				0.58	**0.001**				0.74	**0.001**				0.44	**0.001**				0.15	0.198			
N	0.04	0.070				0.20	0.067				0.15	0.165				0.08	0.369				0.13	0.211				0.14	0.195			
C	0.03	0.175				0.11	0.250				0.04	0.600				0.03	0.749				0.09	0.344				0.25	0.052			
C/N	0.06	**0.031**				0.29	**0.021**				0.27	**0.030**				0.09	0.334				0.07	0.455				0.06	0.501			
pH	0.52	**0.001**				0.65	**0.001**				0.42	**0.005**				0.51	**0.003**				0.37	**0.007**				0.35	**0.010**			
ergosterol	0.27	**0.001**				0.21	0.069				0.34	**0.010**				0.72	**0.001**				0.57	**0.001**				0.14	0.215			

Table 1 Results of PERMANOVA tests and correlations between wood properties and the NMDS analyses, based on Bray-Curtis dissimilarity matrix built from the OTU sequence relative abundances, for the whole model considering all samples and for each tree species independently. Values in bold are significant at $P < 0.05$.

Globally, the dominant phyla were Ascomycota (50%) and Basidiomycota (49%), which had similar proportions over time and regardless of the tree species considered (Supplementary data 7, 8). By examining in more detail fungal community composition, the most striking effect was the heterogeneity in the succession of fungal taxa (Figure 3A, Supplementary data 8). Among the 32 most abundant genera represented by at least 5% in at least one treatment, some genera, such as *Mycena* or *Camarops*, were present in all tree species and across all age classes but with different proportions and no clear pattern. However, some genera, such as *Auricularia*, were more abundant in some tree species, e.g., *Acer campestre* and *Ulmus laevis*, and in the young dead wood. *Eutypa* was highly abundant in *Acer campestre* in age classes 2-4 but absent or scarce in other treatments. Most of the other genera were specific to certain tree species combined with certain age classes (Figure 3A, Supplementary data 8).

The putative ecological classification of the genera demonstrated the dominance of saprotrophs (53% of all sequences) and white-rot fungi (25%), followed by plant pathogens (6%) and ectomycorrhizal fungi (2%). The share of soft-rot and brown-rot fungi was even rarer (Figure 3B). Plant pathogens were particularly abundant in *Acer campestre*. Ectomycorrhizal fungi were present in all treatments, including the youngest dead wood, while fungal parasites and decomposers were more abundant in age classes 1 and 2. Of the brown-rot fungi, 87% were recorded in *Quercus robur*.

Among the potential drivers of fungal community composition, variation partitioning analyses showed that the pure effects of chemistry, tree species and age class were significant. Most of the variation was explained by wood chemistry, i.e., the content of C, N, water and pH (3.4%), as well as tree species (3.2%) and their combination (1.0%). Dead wood age explained 0.6% of the variation. Within individual tree species, pure effects of wood chemistry and age were significant in *Acer campestre*, *Fraxinus angustifolia* and *Quercus*

robur, while *Carpinus betulus* only showed a pure effect of chemistry; neither chemistry nor age effect was significant in *Ulmus laevis*.

Among the environmental variables, pH, water content and age class were the most important factors driving fungal community structure, followed by C/N ratio (Table 1, Figure 2). Regardless of the tree species considered, pH had a significant effect on fungal community structure (Table 1, Supplementary data 6). Except for pH, no other wood properties were significant for *Ulmus laevis*. For the other tree species, water content played a significant role together with age class and C/N ratio depending on the tree species (Table 1, Supplementary data 6).

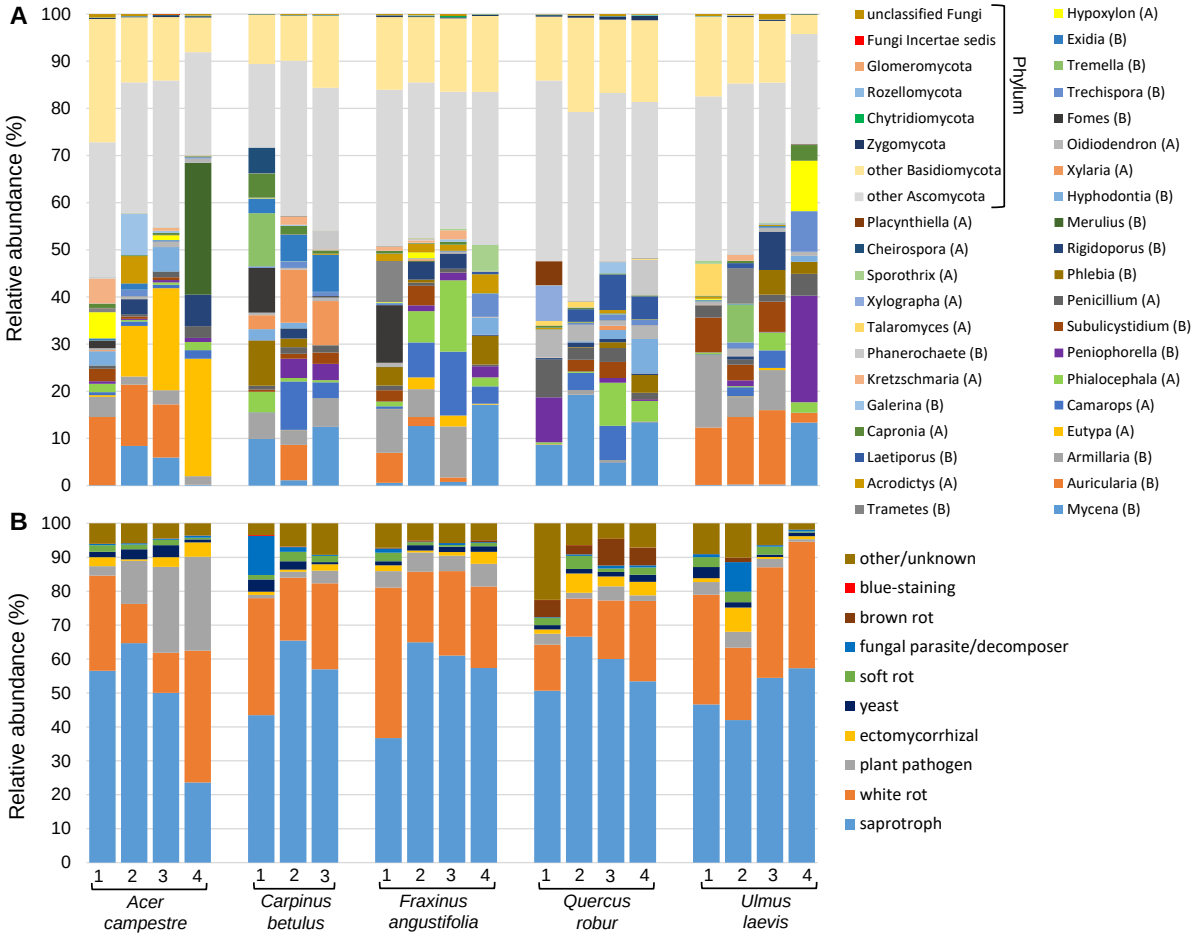

Fig. 3. Fungi occurring in decomposing dead wood in the natural floodplain forest. The data represent the means of relative abundances for (A) genera and (B) potential ecology. Abbreviations: A: Ascomycota, B: Basidiomycota. Numbers indicate the age class of the dead wood. In panel (A), only genera with at least 5% relative abundance in one of the treatments are specified, and all others are classified to the phylum rank.

3.3. Specificity level of the fungal community in dead wood

Mantel correlation analyses between the fungal community dissimilarity and time distance matrices showed significantly increasing dissimilarities with increasing time for *Fraxinus angustifolia*, *Ulmus laevis* and *Quercus robur*; the rate of change in community composition was fastest in *Fraxinus angustifolia* and slowest in *Quercus robur* (Figure 4).

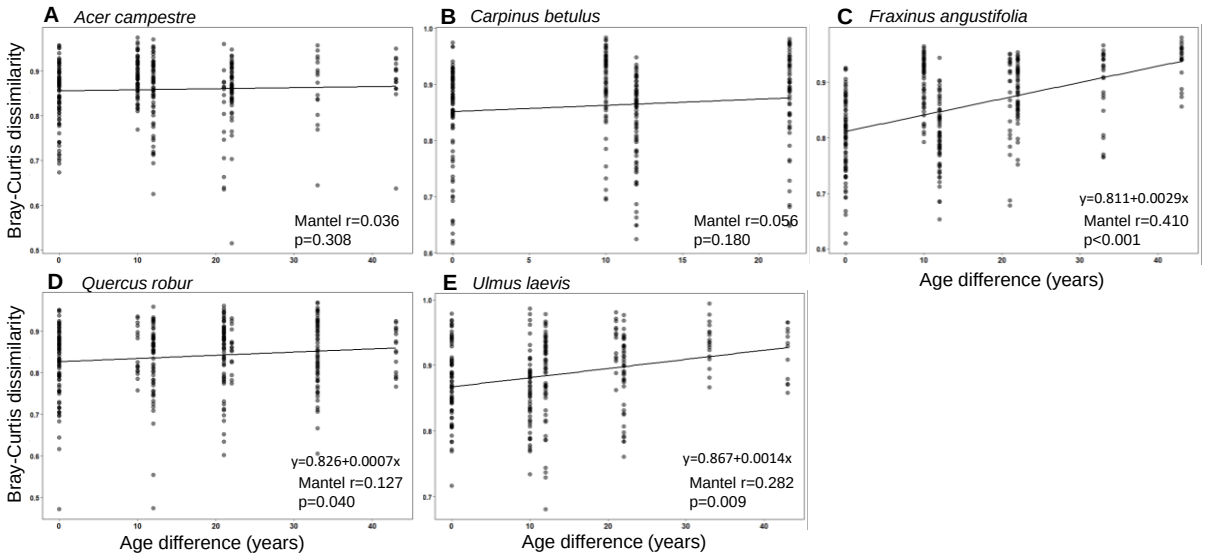

Fig. 4. Successional change in fungal community composition of the natural floodplain forest. The plots represent the correlations of Bray-Curtis dissimilarity of dead wood fungal communities and dead wood age class distances. Mantel correlation tests indicate the significance of the correlation between the Bray-Curtis dissimilarity matrix and the age matrix.

Among the 220 abundant OTUs, 55 taxa were found in all five tree species, 51 in four tree species, 54 in three tree species, and 43 in two tree species. Only 17 OTUs were found exclusively on a single tree species. Of these, 12 were found in *Quercus robur* (*Dactylospora sp.* (a lichen parasite), *Sodiomyces sp.*, *Cladophialophora sp.*, *Sphaeropsis sp.*, *Rhodoveronaea sp.*, *Sorocybe sp.*, *Oidiodendron sp.*, *Mortierella parvispora*, *Mycena sp.* (the last eight taxa representing saprotrophs), *Scytalidium sp.* (a soft-rot fungus), and two putative white-rot fungi, i.e., *Hymenochaete rubiginosa* and *Piloporia sp.*), three in *Acer campestre* (the plant pathogen, *Eutypa sp.*, and two saprotrophs, *Strossmayeria basitricha* and *Waitea*

sp.) and two in *Carpinus betulus* (*Stereum hirsutum*, a white-rot fungus, and *Exophiala sp*., a saprotroph). Of the 220 abundant OTUs, 167 were recorded in *Ulmus laevis*, 164 in *Fraxinus angustifolia*, 147 in *Acer campestre*, 136 in *Quercus robur* and 130 in *Carpinus betulus* (Supplementary data 9).

Fungi showed distinct associations with dead wood of certain ages, reflecting their position in succession; for example, *Fomes fomentarius* and *Phlebia acerina* were mostly found in recently dead wood, while *Mycena inclinata* and *Hyphodontia pallidula* were found mostly in wood at age classes 3 and 4 (Supplementary data 9). The fungi with a wider range of tree hosts were significantly more abundant in dead wood of the middle age classes (R^2 = 0.062, $P < 0.001$; Figure 5), while specialist fungi were most common in the youngest and oldest dead wood.

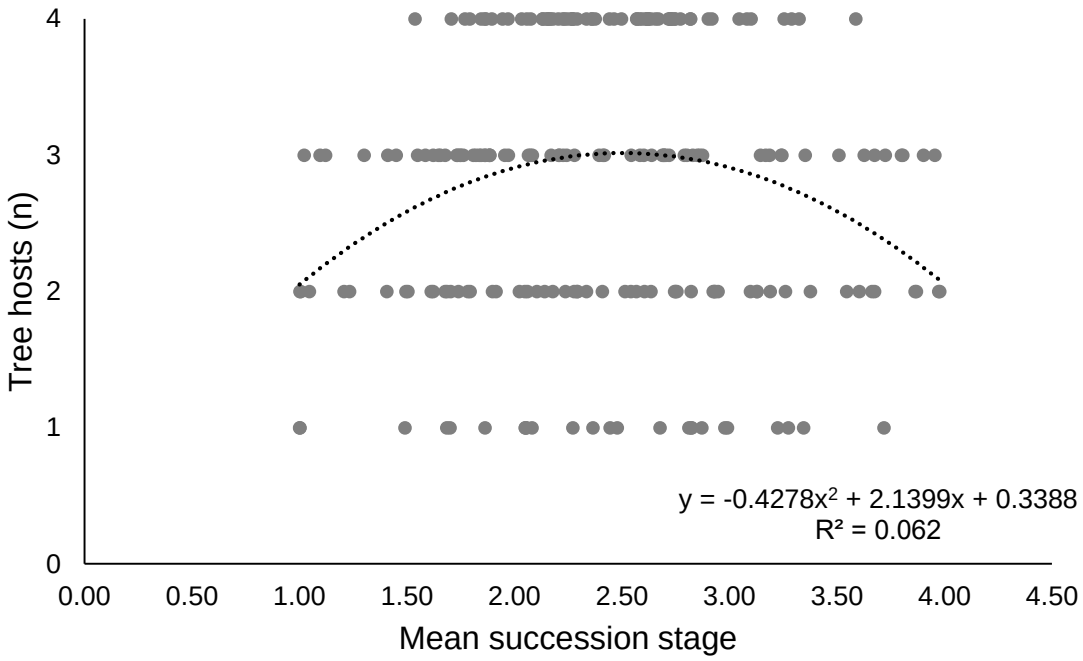

Fig. 5. Number of trees from the natural floodplain forest hosting fungi, and showing stages of succession on dead wood. Only the OTUs represented by at least 0.5% sequence relative abundance for at least three individual samples were considered. *Carpinus betulus* was excluded from the analysis since no CWD of age class 4 was recorded.

4. Discussion

Due to anthropogenic activities, unmanaged old forests become rare and remain largely understudied. This is even more the case of floodplain forests. Our study highlighted the high diversity of wood-associated fungi in this ecosystem. We demonstrated that assembly of dead wood fungal communities was largely stochastic but showed clear successional patterns with fungal taxa specific to early or late stages of wood decay. The majority of fungi showed low specificity and inhabited wood of multiple tree species while tree species-specific fungi were more abundant in early and late decomposition stages. The effects of decomposition time on the changes in wood properties and fungal community diversity were limited while tree species and wood chemistry had significant effects on fungal diversity, structure and composition. The wood chemistry, mainly pH, was the main factor governing the fungal composition.

4.1. Dead wood properties along the decomposition process

Although wood properties showed little consistency in development over time, the significant trends observed were consistent with other tree species (Fukasawa et al. 2009, Baldrian et al. 2016). Thus, *Fraxinus angustifolia* combined an increase in water content and N and a decrease in pH. The only other tree species that showed a significant effect was *Ulmus laevis*, for which pH increased with time. The water content in fresh dead wood from age class 1, ranging from 43% to 66% on average, was very high compared with the values obtained for seven broadleaf tree species in another European temperate forest (Purahong et al. 2018c). This difference can be explained by the floodplain nature of the Ranšpurk natural forest (Unar and Šamonil 2008) and places the dead wood decomposition in a different context where limitations from wood drying probably did not exist. Contrary to what has been demonstrated in other dead wood studies (Köster et al. 2015, Baldrian et al. 2016, Tláskal et

438 al. 2017), the N and C contents and C/N ratios did not change a lot during decomposition.
439 There was large variation among tree species, as noted previously (Purahong et al. 2018c). pH
440 varied between tree species, as found previously (Kahl et al. 2017), and increased
441 acidification during dead wood decomposition was consistent with previous studies
442 (Eichlerová et al. 2015, Baldrian et al. 2016, Tláskal et al. 2017). It is important to note that
443 the weak response of wood chemistry over decomposition time might indicate a high variation
444 among individual CWD samples and the differences in decomposition rates among trees.
445 Vrška et al. (2015) demonstrated that *Carpinus betulus* in the same forest needed an average
446 of 16 years to reach 50% disintegration, while *Fraxinus angustifolia* reached the same value
447 after 22 years, but the variation in these numbers was very high.
448 A variation of fungal biomass during wood decomposition was observed for two of the
449 five tree species. The lower fungal biomass during early decomposition observed in *Carpinus*
450 *betulus* and *Fraxinus angustifolia* could be partly due to the physical properties of the young
451 wood of these species. Indeed, Kahl et al. (2017) showed that *Fraxinus* sp. and *Carpinus*
452 *betulus* had the highest wood density compared with *Quercus* sp. and *Acer* sp. In addition,
453 Noll et al. (2016) demonstrated a positive correlation between fungal biomass and N content,
454 which could partly explain the low fungal biomass in logs of age class 1 for these two species
455 that had the lowest average N contents in this age class. The subsequent increase in fungal
456 biomass in later decomposition stages, up to fivefold in the case of *Fraxinus angustifolia*, was
457 followed by a slight decrease. This higher fungal biomass in the late stages compared with the
458 early decomposition stages was in accordance with the development in *Fagus sylvatica*, *Picea*
459 *abies* and *Abies alba* (Baldrian et al. 2016). In addition, a slight decrease in fungal biomass in
460 the later stages was visible after 22 years of decomposition in *Carpinus betulus* and after 44
461 years in *Fraxinus angustifolia*. This could be explained by the high complexity of the C

462 compounds in the latest stages of decomposition that provide little available nutrients to fungi,
463 as is the case with other types of litter (Bani et al. 2018, Štursová et al. 2020b).
464 Among the potential drivers that governed the composition of fungal communities,
465 chemistry played the primary role, explaining most of the variance in composition, for all tree
466 species except *Ulmus laevis*. More precisely, water content, pH and C/N ratio were the most
467 important drivers. Indeed, water is an essential element regulating the activity of wood fungal
468 communities (Błońska et al. 2019, Rinne-Garmston et al. 2019), and their composition, as
469 previously demonstrated in soil (Kaisermann et al. 2015) and litter (Sherman et al. 2014). pH
470 affects multiple aspects of fungal ecophysiology, including mycelial growth and sporulation
471 (Gorai and Sharma 2018) and the activity of wood-degrading fungal enzymes (Baldrian 2006,
472 Štursová et al. 2020b), and has been found to be the main driver of fungal community
473 composition in broadleaved tree species (Purahong et al. 2018c). Finally, C/N ratio is a
474 fundamental element promoting fungal growth, activity and community composition
475 (Baldrian et al. 2016).
476
477 *4.2. Drivers of fungal community composition in dead wood according to tree species*
478 Our results identified tree species as the main factor driving the composition of the
479 fungal communities. Although some differences in the fungal species diversity were observed,
480 with *Quercus robur* having the highest diversity and *Carpinus betulus* and *Ulmus laevis*
481 having the lowest diversity, no links to wood properties were evident. Thus, the fungal species
482 diversity seems tree-specific, which could suggest the importance of the initial fungal
483 community composition inherent to the tree species in the succession of fungal communities
484 in dead wood (Boddy 2000, Krah et al. 2018).
485 It should be noted that, contrary to what was expected, fungal diversity did not
486 increase during succession. However, the composition of fungal communities varied with age

class and consisted of a distinction between the oldest and youngest age classes. Both physical and chemical changes within wood and larger scale environmental factors can affect the composition of dead wood communities (Błońska et al. 2019, Moreno-Fernández et al. 2020). The difference in fungal composition between the oldest and youngest age classes was most prominent in *Quercus robur* and *Fraxinus angustifolia*, which are the two species with the longest residence times compared with *Acer campestre*, *Carpinus betulus* and *Ulmus* sp (Vrška et al. 2015). Thus, for these two species, the last stage of decomposition, after 44 years, involves a specific community that shows very little overlap with the initial community. In line with this, *Quercus robur* and *Fraxinus angustifolia* tended to have distinct fungal community compositions from each other and from the three other tree species. This distinction in fungal community composition was consistent with Purahong et al. (2018a), who studied *Quercus* sp., *Fraxinus* sp. and *Carpinus* sp.

Our study confirmed the predominance of Ascomycota and Basidiomycota in dead wood (Baldrian et al. 2016, Purahong et al. 2018c), but in equivalent proportions during the entire course of succession. This is rather exceptional, because Ascomycota were generally dominant compared with Basidiomycota, especially during the early stages of decomposition (Baldrian et al. 2016, Purahong et al. 2018b), and may perhaps be linked to the high water content in the dead wood in our study. In the Ranšpurk floodplain forest, among the 32 most abundant genera, 65% were Basidiomycota and 35% were Ascomycota. Half of the total abundance of these 32 most abundant genera was represented by only five genera: *Mycena*, *Auricularia*, *Armillaria*, *Eutypa* and *Camarops*. They are all classified as saprotrophs except *Eutypa*, known as a plant pathogen (Carter 1991, Hendry et al. 1993, Rolshausen et al. 2006) that can also be saprotrophic (Heilmann-Clausen and Boddy 2005) depending on climatic conditions, as low moisture content (Hendry et al. 1998). This could explain its presence, almost exclusively, in *Acer campestre* (91%), that was the tree species exhibiting the lowest

values of water content, which coincided with the logs containing a high abundance of *Eutypa*. *Armillaria* is a white-rot basidiomycete, previously identified as dominant in dead wood of *Quercus*, *Fraxinus* and *Carpinus* in a European temperate forest (Purahong et al. 2018b), and whose some species are known for their pathogenic role (Guillaumin and Legrand 2013, Kubiak et al. 2017, Sipos et al. 2018). Here, *Armillaria* was present in all decay stages for all tree species, due to both its saprotrophic and pathogenic lifestyles.

4.3. Assembly rules of the fungal communities in dead wood

In accordance with our hypothesis, the level of dissimilarities in the composition of the fungal communities was approximately linked to the residence time of the tree species (Vrška et al. 2015). As demonstrated by Vrška et al. (2015) in the same study area, the order was *Quercus robur* > *Fraxinus angustifolia* > *Acer campestre* > *Carpinus betulus* > *Ulmus* sp., indicating that the slowest decomposition was in the first species and the fastest decomposition was in the last species. In agreement with this, the rate of change in fungal community composition over time indicated that fungal species replacements were faster in *Ulmus laevis* and *Fraxinus angustifolia* than in *Quercus robur* (Figure 4).

Quercus robur had the highest proportion of specialist fungi, while *Acer campestre* and *Carpinus betulus* had three and two fungal specialists, respectively. Among the 17 specialist fungal species, only five were Basidiomycota, one belonged to Zygomycota and the rest were Ascomycota, which was consistent with Purahong et al. (2018b), who demonstrated a significantly higher proportion of tree-specific fungi among Ascomycota. The highest specificity level found in *Quercus robur* could be partly due to its greater phylogenetic distance from the other tree species (Purahong et al. 2018c), as well as the physico-chemical properties of its wood, which was the slowest to decompose (Vrška et al. 2015).

Overall, we obtained a high level of generalist fungal species (25%), and the rest of the taxa were not absolute specialists but were shared by several tree species. Only 8% of the most abundant OTUs were specialists specific to one tree species. This high level of generalist species among the fungal communities decomposing wood has already been highlighted (Baldrian et al. 2016) and may be typical in natural forests where multiple tree species grow together. In addition, we demonstrated that the level of tree specificity decreased in the middle stages of decomposition. This was probably a consequence of the gradual colonization of dead wood by soil fungi (López-Mondéjar et al. 2018, Šťursová et al. 2020b) and spore deposition (Edman et al. 2004), or the loss of distinct microhabitats together with the specific fungi colonizing these specific microhabitats (Juutilainen et al. 2011). Interestingly, the level of specialization increased again in late stages of decomposition. We can hypothesize that old dead wood might still keep some legacy of its former origin, as suggested by Weslien et al. (2011) – in contrast to plant litter (Šťursová et al. 2020b). We could also suppose that the changes in the wood chemistry specific to each tree species lead to the formation of tree-specific complex compounds that select for specific fungal taxa (Kahl et al. 2017).

We have implemented and optimized in house standard protocols to remove most of carry over DNA between samples during sample collection and processing. Moreover, to detect sample-to-sample carry over, we searched for highly abundant fungal taxa across all samples and recorded high share of samples with zero observations, indicating that sample-to-sample carry over is very limited, although it cannot be fully excluded as in any high-throughput sequencing effort. The removal of singletons and the use of thresholds of relative abundance further helped to limit the potential effect of such issues on the results. Negative control samples may be used as an alternative approach to this issue, but such samples taken at different steps of wood processing (e.g., drill bit washes, mill washes) failed to amplify in

our tests. The use of positive controls (i.e., mock communities) would be another alternative to confirm the absence of carry over.

It should be noted that we used read numbers as estimate to measure the relative abundances of fungal taxa. Such a method is prone to PCR biases (Palmer et al. 2018). In contrast, the use of presence-absence of fungal taxa to characterize fungal communities is less prone to such biases but has the potential to rate rare taxa on the same level as more abundant ones (Palmer et al. 2018) which is especially disturbing in datasets where certain species highly dominate, such as in the deadwood. In the present study, we analysed fungal communities in both ways and the presence-absence based results are contained in the Supplemental data 2-4.

5. Conclusions

The present study demonstrated a high level of fungal diversity in a Central European floodplain forest with a high diversity of trees and high moisture content. The assembly of dead wood communities under these conditions shows a high level of stochasticity with little consistency in the development of dead wood chemistry over time. Nevertheless, tree species and wood chemistry, in particular pH, are the most important drivers of fungal community composition, although the share of unexplained variation remains high. The rate of successional change in fungal communities reflects the rate of wood decomposition. This study highlights the importance of dead wood and tree species diversity in preserving the biodiversity of fungi.

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585

586 **References**

587 Arnstadt, T., Hoppe, B., Kahl, T., Kellner, H., Krüger, D., Bauhus, J., Hofrichter, M., 2016.
588 Dynamics of fungal community composition, decomposition and resulting deadwood
589 properties in logs of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris*. For. Ecol.
590 Manag. 382, 129-142.

591 Baldrian, P., 2006. Fungal laccases–occurrence and properties. FEMS Microbiol. Rev. 30,
592 215-242.

593 Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol.
594 Rev. 41, 109-130.

595 Baldrian, P., Větrovský, T., Lepinay, C., Kohout, P., 2021. High-throughput sequencing view
596 on the magnitude of global fungal diversity. Fungal Diversity in press, doi:
597 10.1007/s13225-021-00472-y.

598 Baldrian, P., Zrůstová, P., Tláskal, V., Davidová, A., Merhautová, V., Vrška, T., 2016. Fungi
599 associated with decomposing deadwood in a natural beech-dominated forest. Fungal
600 Ecol. 23, 109-122.

601 Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., Tonon, G., Brusetti,
602 L., 2018. The role of microbial community in the decomposition of leaf litter and
603 deadwood. Appl. Soil Ecol. 126, 75-84.

604 Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit,
605 P., Sánchez-García, M., Ebersberger, I., de Souza, F., Amend, A.S., Jumpponen, A.,
606 Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson,
607 K.M., Vik, U., Veldre, V, Nilsson, R.H., 2013. Improved software detection and
608 extraction of ITS1 and ITS 2 from ribosomal ITS sequences of fungi and other

609 eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914-
610 919.

611 Blaser, S., Prati, D., Senn-Irlet, B., Fischer, M., 2013. Effects of forest management on the
612 diversity of deadwood-inhabiting fungi in Central European forests. For. Ecol. Manag.
613 304, 42-48.

614 Błońska, E., Kacprzyk, M., Spolnik, A., 2017. Effect of deadwood of different tree species in
615 various stages of decomposition on biochemical soil properties and carbon storage.
616 Ecol. Res. 32, 193-203.

617 Błońska, E., Lasota, J., Piaszczyk, W., 2019. Carbon and nitrogen stock in deadwood biomass
618 in natural temperate forest along a soil moisture gradient. Plant Biosyst. 154, 213-221.

619 Boddy, L., 2000. Interspecific combative interactions between wood-decaying
620 basidiomycetes. FEMS Microbiol. Ecol. 31, 185-194.

621 Boddy, L., Hiscox, J., 2016. Fungal ecology: principles and mechanisms of colonization and
622 competition by saprotrophic fungi. Microbiol. Spectr. 4, 4-6.

623 Carter, M.V., 1991. The status of *Eutypa lata* as a pathogen. CAB International, Wallingford.

624 De Meo, I., Lagomarsino, A., Agnelli, A.E., Paletto, A., 2019. Direct and indirect assessment
625 of carbon stock in deadwood: Comparison in Calabrian Pine (*Pinus brutia* Ten. subsp.
626 *brutia*) forests in Italy. For. Sci. 65, 460-468.

627 Doerfler, I., Müller, J., Gossner, M.M., Hofner, B., Weisser, W.W., 2017. Success of a
628 deadwood enrichment strategy in production forests depends on stand type and
629 management intensity. For. Ecol. Manag. 400, 607-620.

630 Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads.
631 Nat. Methods 10, 996.

632 Edman, M., Kruys, N., Jonsson, B.G., 2004. Local dispersal sources strongly affect
633 colonization patterns of wood-decaying fungi on spruce logs. Ecol. Appl. 14, 893-901.

634 Eichlerová, I., Homolka, L., Žifčáková, L., Lisá, L., Dobiášová, P., Baldrian, P., 2015.
635 Enzymatic systems involved in decomposition reflects the ecology and taxonomy of
636 saprotrophic fungi. Fungal Ecol. 13, 10-22.

637 Franklin, R.B., Mills, A.L., 2009. Importance of spatially structured environmental
638 heterogeneity in controlling microbial community composition at small spatial scales
639 in an agricultural field. Soil Biol. Biochem. 41, 1833-1840.

640 Fukasawa, Y., Osono, T., Takeda, H., 2009. Dynamics of physicochemical properties and
641 occurrence of fungal fruit bodies during decomposition of coarse woody debris of
642 *Fagus crenata*. J. For. Res. 14, 20-29.

643 Goodell, B., Qian, Y., Jellison, J., 2008. Fungal decay of wood: soft rot-brown rot-white rot.
644 In: Schultz, T.P., Militz, H., Freeman, M.H., Goodell, B., Nicholas, D.D. (Eds.),
645 Development of Commercial Wood Preservatives. ACS Symposium Series Volume
646 982, pp. 9-31.

647 Goodell, B., Winandy, J.E., Morrell, J.J., 2020. Fungal degradation of wood: Emerging data,
648 new insights and changing perceptions. Coatings 10, 1210.

649 Gorai, B., Sharma, R., 2018. Determination of optimum temperature and pH for mycelial
650 growth of *Pleurotus* spp/strains. Int. J. Microbiol. Res. 10, 1287-1289.

651 Griffiths, H.M., Ashton, L.A., Evans, T.A., Parr, C.L., Eggleton, P., 2019. Termites can
652 decompose more than half of deadwood in tropical rainforest. Curr. Biol. 29, R118-
653 R119.

654 Guillaumin, J.J., Legrand, P., 2013. Armillaria root rots. In: Gonthier, P., Nicolotti, G. (Eds.),
655 Infectious Forest Diseases. CAB International, Oxfordshire, pp.159-177.

656 Heilmann-Clausen, J., Boddy, L., 2005. Inhibition and stimulation effects in communities of
657 wood decay fungi: exudates from colonized wood influence growth by other species.
658 Microb. Ecol. 49, 399-406.

659 Hekkala, A.M., Ahtikoski, A., Päätalo, M.L., Tarvainen, O., Siipilehto, J., Tolvanen, A.,
660 2016. Restoring volume, diversity and continuity of deadwood in boreal forests.
661 Biodivers. Conserv. 25, 1107-1132.

662 Hendry, S.J., Boddy, L., Lonsdale, D., 1993. Interactions between callus cultures of European
663 beech, indigenous ascomycetes and derived fungal extracts. New Phytol. 123, 421-
664 428.

665 Hendry, S.J., Lonsdale, D., Boddy, L., 1998. Strip-cankering of beech (*Fagus sylvatica*):
666 Pathology and distribution of symptomatic trees. New Phytol. 140, 549-565.

667 Hiscox, J., Savoury, M., Müller, C.T., Lindahl, B.D., Rogers, H.J., Boddy, L., 2015. Priority
668 effects during fungal community establishment in beech wood. ISME J. 9, 2246-2260.

669 Hoppe, B., Purahong, W., Wubet, T., Kahl, T., Bauhus, J., Arnstadt, T., Hofrichter, M.,
670 Buscot, F., Krüger, D., 2016. Linking molecular deadwood-inhabiting fungal diversity
671 and community dynamics to ecosystem functions and processes in Central European
672 forests. Fungal Divers. 77, 367-379.

673 Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid,
674 Y., Stendil, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New
675 primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial
676 and natural communities. FEMS Microbiol. Ecol. 82, 666-677.

677 Jacobs, J.M., Work, T.T., 2012. Linking deadwood-associated beetles and fungi with wood
678 decomposition rates in managed black spruce forests. Can. J. For. Res. 42, 1477-1490.

679 Juutilainen, K., Halme, P., Kotiranta, H., Mönkkönen, M., 2011. Size matters in studies of
680 dead wood and wood-inhabiting fungi. Fungal Ecol. 4, 342-349.

681 Kahl, T., Arnstadt, T., Baber, K., Bässler, C., Bauhus, J., Borken, W., Buscot, F., Floren, A.,
682 Heibl, C., Hessenmöller, D., Hofrichter, M., Hoppe, B., Kellner, H., Krüger, D.,
683 Linsenmair, K.E., Matzner, E., Otto, P., Purahong, W., Seilwinder, C., Schulze, E-D.,
684 Wende, B., Weisser, W.W., Gossner, M.M., 2017. Wood decay rates of 13 temperate
685 tree species in relation to wood properties, enzyme activities and organismic
686 diversities. For. Ecol. Manag. 391, 86-95.

687 Kaisermann, A., Maron, P.A., Beaumelle, L., Lata, J.C., 2015. Fungal communities are more
688 sensitive indicators to non-extreme soil moisture variations than bacterial
689 communities. Appl. Soil Ecol. 86, 158-164.

690 Köster, K., Metslaid, M., Engelhart, J., Köster, E., 2015. Dead wood basic density, and the
691 concentration of carbon and nitrogen for main tree species in managed hemiboreal
692 forests. For. Ecol. Manag. 354, 35-42.

693 Krah, F.S., Seibold, S., Brandl, R., Baldrian, P., Müller, J., Bässler, C., 2018. Independent
694 effects of host and environment on the diversity of wood-inhabiting fungi. J. Ecol.
695 106, 1428-1442.

696 Kubiak, K., Żółciak, A., Damszel, M., Lech, P., Sierota, Z., 2017. *Armillaria* pathogenesis
697 under climate changes. Forests 8, 100.

698 Larrieu, L., Cabanettes, A., Gonin, P., Lachat, T., Paillet, Y., Winter, S., Bouget, C.,
699 Deconchat, M., 2014. Deadwood and tree microhabitat dynamics in unharvested

temperate mountain mixed forests: a life-cycle approach to biodiversity monitoring. For. Ecol. Manag. 334, 163-173.

Lassauce, A., Paillet, Y., Jactel, H., Bouget, C., 2011. Deadwood as a surrogate for forest biodiversity: meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms. Ecol. Indic. 11, 1027-1039.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. New Phytol. 199, 288-299.

López-Mondéjar, R., Brabcová, V., Štursová, M., Davidová, A., Jansa, J., Cajthaml, T., Baldrian, P., 2018. Decomposer food web in a deciduous forest shows high share of generalist microorganisms and importance of microbial biomass recycling. ISME J. 12, 1768-1778.

Luyssaert, S., Schulze, E.D., Börner, A., Knohl, A., Hessenmöller, D., Law, B.E., Ciais, P., Grace, J., 2008. Old-growth forests as global carbon sinks. Nature 455, 213-215.

Moreno-Fernández, D., Hernández, L., Cañellas, I., Adame, P., Alberdi, I., 2020. Analyzing the dynamics of the deadwood carbon pool in Spain through the European Level I Monitoring Programme. For. Ecol. Manag. 463, 118020.

Müller, J., Ulyshen, M., Seibold, S., Cadotte, M., Chao, A., Bässler, C., Vogel, S., Hagge, J., Weiß, I., Baldrian, P., Tláskal, V., Thorn, S., 2020. Primary determinants of communities in deadwood vary among taxa but are regionally consistent. Oikos 129, 1579-1588.

722 Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel,
723 D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U.,
724 Abarenkov, K., 2019. The UNITE database for molecular identification of fungi:
725 handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47,
726 D259-D264.

727 Noll, L., Leonhardt, S., Arnstadt, T., Hoppe, B., Poll, C., Matzner, E., Hofrichter, M., Kellner,
728 H., 2016. Fungal biomass and extracellular enzyme activities in coarse woody debris
729 of 13 tree species in the early phase of decomposition. For. Ecol. Manag. 378, 181-
730 192.

731 Nordén, J., Abrego, N., Boddy, L., Bässler, C., Dahlberg, A., Halme, P., Hällfors, M.,
732 Maurice, S., Menkis, A., Miettinen, O., Mäkipää, R., Ovaskainen, O., Penttilä, R.,
733 Saine, S., Snäll, T., Junninen, K., 2020. Ten principles for conservation translocations
734 of threatened wood-inhabiting fungi. Fungal Ecol. 44, 100919.

735 Odriozola, I., Abrego, N., Tláskal,V., Zrůstová, P., Morais, D., Větrovský, T.,
736 Ovaskainen,O., Baldrian, P., 2020. Fungal communities are important determinants of
737 bacterial community composition in deadwood. mSystems 5, e01017-20.

738 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin,
739 P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner,
740 H., 2016. Vegan: Community Ecology Package. R package version 2.4-3. Vienna: R
741 Foundation for Statistical Computing.

742 Paillet, Y., Bergès, L., Hjältén, J., Ódor, P., Avon, C., Bernhardt-Römermann, M., Bijlsma, R-
743 J., De Bruyn, L., Fuhr, M., Grandin, U., Kanka, R., Lundin, L., Luque, S., Magura, T.,
744 Matesanz, S., Mészáros, I., Sebastià, M-T., Schmidt, W., Standovár, T., Tóthmérész,
745 B., Uotila, A., Valladares, F., Vellak, K., Virtanen, R., 2010. Biodiversity differences

between managed and unmanaged forests: meta-analysis of species richness in Europe. Conserv. Biol. 24, 101-112.

Palmer, J.M., Jusino, M.A., Banik, M.T., Lindner, D.L., 2018. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6, e4925.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333, 988-993.

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S.T., Baldrian, P., Frøslev, T.G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., Liu, J.-K., Pawlowska, J., Põldmaa, K., Pärtel, K., Runnel, K., Hansen, K., Larsson, K.-H., Hyde, K.D., Sandoval-Denis, M., Smith, M.E., Toome-Heller, M., Wijayawardene, N.N., Menolli, N., Reynolds, N.K., Drenkhan, R., Maharachchikumbura, S.S.N., Gibertoni, T.B., Læssøe, T., Davis, W., Tokarev, Y., Corrales, A., Soares, A.M., Agan, A., Machado, A.R., Argüelles-Moyao, A., Detheridge, A., de Meiras-Ottoni, A., Verbeken, A., Dutta, A.K., Cui, B.-K., Pradeep, C.K., Marín, C., Stanton, D., Gohar, D., Wanasinghe, D.N., Otsing, E., Aslani, F., Griffith, G.W., Lumbsch, T.H., Grossart, H.-P., Masigol, H., Timling, I., Hiiesalu, I., Oja, J., Kupagme, J.Y., Geml, J., Alvarez-Manjarrez, J., Ilves, K., Loit, K., Adamson, K., Nara, K., Küngas, K., Rojas-Jimenez, K., Bitenieks, K., Irinyi, L., Nagy, L.L., Soonvald, L., Zhou, L.-W., Wagner, L., Aime, M.C., Öpik, M., Mujica, M.I., Metsoja, M., Ryberg, M., Vasar, M., Murata, M., Nelsen, M.P., Cleary, M., Samarakoon, M.C., Doilom, M., Bahram, M., Hagh-Doust, N., Dulya, O., Johnston, P., Kohout, P., Chen, Q., Tian, Q., Nandi, R., Amiri, R.,

771 Perera, R.H., dos Santos Chikowski, R., Mendes-Alvarenga, R.L., Garibay-Orijel, R.,
772 Gielen, R., Phookamsak, R., Jayawardena, R.S., Rahimlou, S., Karunarathna, S.C.,
773 Tibpromma, S., Brown, S.P., Sepp, S.-K., Mundra, S., Luo, Z.-H., Bose, T., Vahter,
774 T., Netherway, T., Yang, T., May, T., Varga, T., Li, W., Coimbra, V.R.M., de
775 Oliveira, V.R.T., de Lima, V.X., Mikryukov, V.S., Lu, Y., Matsuda, Y., Miyamoto,
776 Y., Kõljalg, U., Tedersoo, L., 2020. FungalTraits: a user-friendly traits database of
777 fungi and fungus-like stramenopiles. Fungal Diversity 105, 1-16.

778 Přívětivý, T., Baldrian, P., Šamonil, P., Vrška, T., 2017. Deadwood density and moisture
779 variation in a natural temperate Spruce-Fir-Beech forest. Preprints 2017050215.

780 Přívětivý, T., Janík, D., Unar, P., Adam, D., Král, K., Vrška, T., 2016. How do environmental
781 conditions affect the deadwood decomposition of European beech (*Fagus sylvatica*
782 L.)?. For. Ecol. Manag. 381, 177-187.

783 Purahong, W., Wubet, T., Krüger, D., Buscot, F., 2018a. Application of next-generation
784 sequencing technologies to conservation of wood-inhabiting fungi. Conserv. Biol. 33,
785 716-724.

786 Purahong, W., Wubet, T., Krüger, D., Buscot, F., 2018b. Molecular evidence strongly
787 supports deadwood-inhabiting fungi exhibiting unexpected tree species preferences in
788 temperate forests. ISME J. 12, 289-295.

789 Purahong, W., Wubet, T., Lentendu, G., Hoppe, B., Jariyavidyanont, K., Arnstadt, T., Baber,
790 K., Otto, P., Kellner, H., Hofrichter, M., Bauhus, J., Weisser, W.W., Krüger, D.,
791 Schulze, E-D., Kahl, T., Buscot, F., 2018c. Determinants of deadwood-inhabiting
792 fungal communities in temperate forests: molecular evidence from a large scale
793 deadwood decomposition experiment. Front. Microbiol. 9, 2120.

794 Rayner, A.D.M., Boddy, L., 1988. Fungal decomposition of wood: its biology and ecology.
795 John Wiley International, Chichester.

796 Rejšek, K., 2007. Acid phosphomonoesterase activity in floodplain forest soils. Soil Water
797 Res. 2, 67-75.

798 Rinne, K.T., Rajala, T., Peltoniemi, K., Chen, J., Smolander, A., Mäkipää, R., 2017.
799 Accumulation rates and sources of external nitrogen in decaying wood in a Norway
800 spruce dominated forest. Funct. Ecol. 31, 530-541.

801 Rinne-Garmston, K.T., Peltoniemi, K., Chen, J., Peltoniemi, M., Fritze, H., Mäkipää, R.,
802 2019. Carbon flux from decomposing wood and its dependency on temperature, wood
803 N_2 fixation rate, moisture and fungal composition in a Norway spruce forest. Glob.
804 Change Biol. 25, 1852-1867.

805 Rolshausen, P.E., Mahoney, N.E., Molyneux, R.J., Gubler, W.D., 2006. A reassessment of the
806 species concept in *Eutypa lata*, the causal agent of *Eutypa* dieback of grapevine.
807 Phytopathology 96, 369-377.

808 Rondeux, J., Sanchez, C., 2010. Review of indicators and field methods for monitoring
809 biodiversity within national forest inventories. Core variable: Deadwood. Environ.
810 Monit. Assess. 164, 617-630.

811 Šamonil, P., Daněk, P., Adam, D., Phillips, J.D., 2017. Breakage or uprooting: How tree death
812 type affects hillslope processes in old-growth temperate forests. Geomorphology 299,
813 76-84.

814 Šamonil, P., Daněk, P., Baldrian, P., Tláskal, V., Tejnecký, V., Drábek, O., 2020.
815 Convergence, divergence or chaos? Consequences of tree trunk decay for pedogenesis
816 and the soil microbiome in a temperate natural forest. Geoderma 376, 114499.

817 Šenhofa, S., Jaunslaviete, I., Šņepsts, G., Jansons, J., Liepa, L., Jansons, Ā., 2020. Deadwood
818 characteristics in mature and old-growth birch stands and their implications for carbon
819 storage. Forests 11, 536.

820 Sherman, C., Grishkan, I., Barness, G., Steinberger, Y., 2014. Fungal community-plant litter
821 decomposition relationships along a climate gradient. Pedosphere 24, 437-449.

822 Shi, G.R., 1993. Multivariate data analysis in palaeoecology and palaeobiogeography - a
823 review. Palaeogeog. Palaeoclimatol. Palaeoecol. 105, 199-234.

824 Siitonen, J., Martikainen, P., Punttila, P., Rauh, J., 2000. Coarse woody debris and stand
825 characteristics in mature managed and old-growth boreal mesic forests in southern
826 Finland. For. Ecol. Manag. 128, 211-225.

827 Sipos, G., Anderson, J.B., Nagy, L.G., 2018. Armillaria. Curr. Biol. 28, R297-R298.

828 Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008.
829 Spatial variability of enzyme activities and microbial biomass in the upper layers of
830 *Quercus petraea* forest soil. Soil Biol. Biochem. 40, 2068-2075.

831 Song, Z., Kennedy, P.G., Liew, F.J., Schilling, J.S., 2017. Fungal endophytes as priority
832 colonizers initiating wood decomposition. Funct. Ecol. 31, 407-418.

833 Stokland, J.N., Siitonen, J., Jonsson, B.G., 2012. Biodiversity of dead wood. Cambridge
834 University Press, Cambridge.

835 Štursová, M., Kohout, P., Human, Z.R., Baldrian, P., 2020a. Production of fungal mycelia in a
836 temperate coniferous forest shows distinct seasonal patterns. J. Fungi 6, 190.

837 Štursová, M., Šnajdr, J., Koukol, O., Tláskal, V., Cajthaml, T., Baldrian, P., 2020b. Long-
838 term decomposition of litter in the montane forest and the definition of fungal traits in
839 the successional space. Fungal Ecol. 46, 100913.

840 Tláskal, V., Brabcová, V., Větrovský, T., Jomura, M., López-Mondéjar, R., Oliveira
841 Monteiro, L.M., Saraiva, J.P., Human, Z.R., Cajthaml, T. da Rocha, U.N., Baldrian,
842 P., 2021. Complementary roles of wood-inhabiting fungi and bacteria facilitate
843 deadwood decomposition. mSystems 6, e01078-20.

844 Tláskal, V., Zrůstová, P., Vrška, T., Baldrian, P., 2017. Bacteria associated with decomposing
845 dead wood in a natural temperate forest. FEMS Microbiol. Ecol. 93, fix157.

846 Ulyshen, M.D., 2016. Wood decomposition as influenced by invertebrates. Biol. Rev. 91, 70-
847 85.

848 Unar, P., Šamonil, P., 2008. The evolution of natural floodplain forests in South Moravia
849 between 1973 and 2005. J. For. Sci. 54, 340-354.

850 Větrovský, T., Baldrian, P., 2015. An in-depth analysis of actinobacterial communities shows
851 their high diversity in grassland soils along a gradient of mixed heavy metal
852 contamination. Biol. Fertil. Soils 51, 827-837.

853 Větrovský, T., Baldrian, P., Morais, D., 2018. SEED 2: a user-friendly platform for amplicon
854 high-throughput sequencing data analyses. Bioinformatics 34, 2292-2294.

855 Větrovský, T., Kolařík, M., Žifčáková, L., Zelenka, T., Baldrian, P., 2016. The *rpb2* gene
856 represents a viable alternative molecular marker for the analysis of environmental
857 fungal communities. Mol. Ecol. Resour. 16, 388-401.

858 Větrovský, T., Morais, D., Kohout, P., Lepinay, C., Algora, C., Awokunle Hollá, S.,
859 Bahnmann, B.D., Bílohnědá, K., Brabcová, V., D'Alò, F., Human, Z.R., Jomura, M.,
860 Kolařík, M., Kvasničková, J., Lladó, S., López-Mondéjar, R., Martinović, T.,
861 Mašínová, T., Meszárošová, L., Michalčíková, L., Michalová, T., Mundra, S.,
862 Navrátilová, D., Odriozola, I., Piché-Choquette, S., Štursová, M., Švec, K., Tláskal,

V., Urbanová, M., Vlk, L., Voříšková, J., Žifčáková, L., Baldrian, P., 2020. GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Sci. Data 7, 228.

Vítková, L., Bače, R., Kjučukov, P., Svoboda, M., 2018. Deadwood management in Central European forests: Key considerations for practical implementation. For. Ecol. Manag. 429, 394-405.

Vrška, T., Adam, D., Hort, L., Odehnalová, P., Horal, D., Král, K., 2006. Developmental dynamics of virgin forest reserves in the Czech Republic II – Floodplain forests – Cahnov-Soutok, Ranšpurk, Jiřina. Academia, Prague, Czech Republic.

Vrška, T., Přívětivý, T., Janík, D., Unar, P., Šamonil, P., Král, K., 2015. Deadwood residence time in alluvial hardwood temperate forests - A key aspect of biodiversity conservation. For. Ecol. Manag. 357, 33-41.

Weslien, J., Djupström, L.B., Schroeder, M., Widenfalk, O., 2011. Long-term priority effects among insects and fungi colonizing decaying wood. J. Anim. Ecol. 80, 1155-1162.

White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Sninsky, D.H., White, T.J. (Eds.), PCR protocols: a guide to methods and applications. Academic Press, London, pp. 315-322.

Zeller, L., Pretzsch, H., 2019. Effect of forest structure on stand productivity in Central European forests depends on developmental stage and tree species diversity. For. Ecol. Manag. 434, 193-204.

884 Zuo, J., Fonck, M., van Hal, J., Cornelissen, J.H.C., Berg, M.P., 2014. Diversity of
885 macrodetritivores in dead wood is influenced by tree species, decay stage and
886 environment. Soil Biol. Biochem. 78, 288-297.

Supplementary data

Successional development of wood-associated fungi associated with dominant tree species in a natural temperate floodplain forest

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Supplementary data 1 Characteristics of the 127 dead wood samples collected.

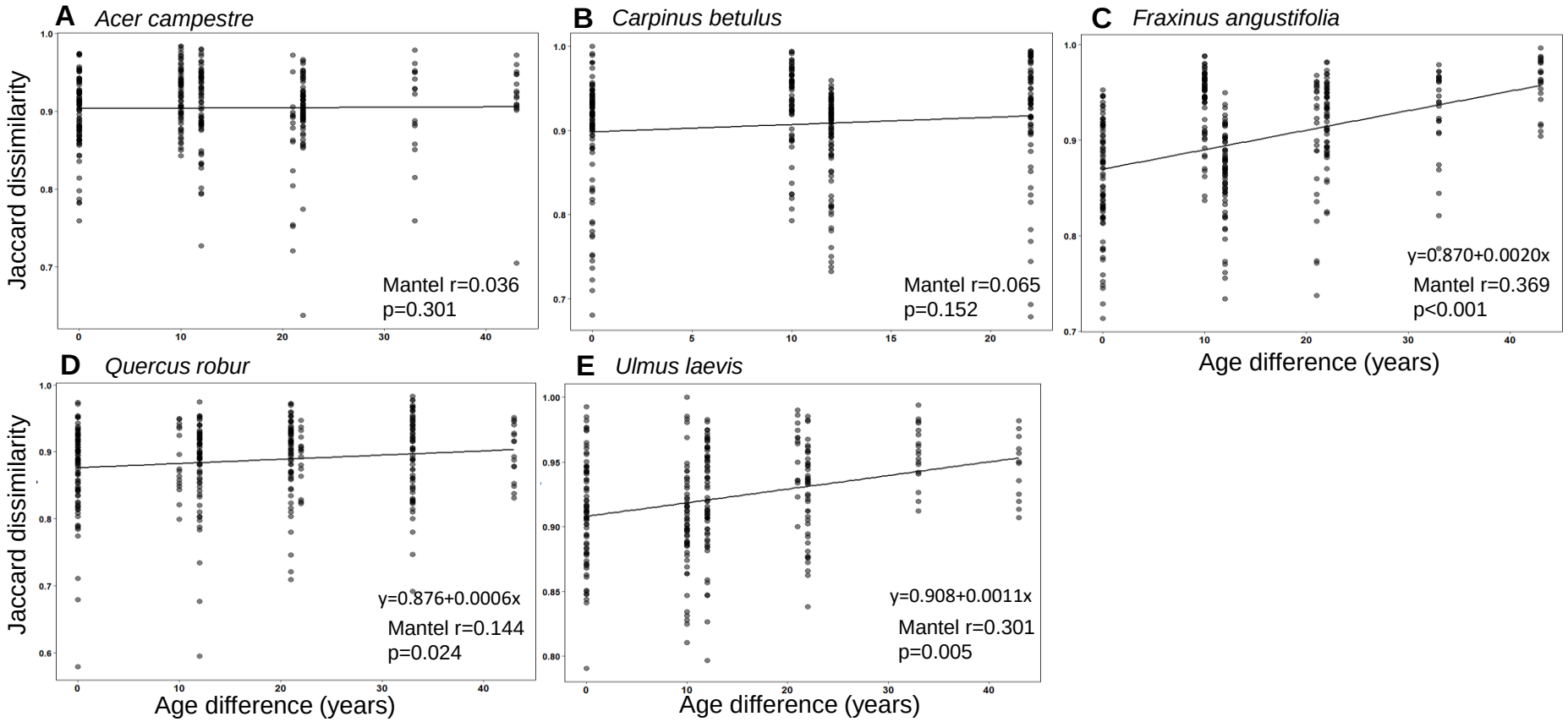
Supplementary data 2 Variation partitioning performed on Jaccard dissimilarity matrix built from the OTU presence-absence table, for the whole model considering all samples and for each tree species independently. Values in bold are significant at $P < 0.05$.

	Whole model				Acer campestre				Carpinus betulus			Fraxinus angustifolia			Quercus robur			Ulmus laevis		
Variables	df	Adjusted R2	F	p	df	Adjusted R2	F	p	Adjusted R2	F	p	Adjusted R2	F	p	Adjusted R2	F	p	Adjusted R2	F	p
year	1	0.013	2.662	**0.001**	1	0.012	1.296	**0.018**	0.007	1.170	0.101	0.029	1.754	**0.001**	0.018	1.456	**0.013**	0.011	1.259	**0.004**
year (chemistry)	_	_	_	_	1	-0.001	0.970	0.594	0.012	1.243	0.096	0.005	1.113	0.248	0.015	1.318	**0.037**	0.003	1.055	0.415
chemistry	5	0.045	2.200	**0.001**	5	0.075	1.408	**0.001**	0.029	1.143	**0.037**	0.091	1.499	**0.001**	0.045	1.236	**0.003**	0.025	1.120	**0.007**
chemistry (year)	_	_	_	_	5	0.062	1.324	**0.001**	0.034	1.164	**0.013**	0.067	1.354	**0.001**	0.042	1.215	**0.003**	0.017	1.078	0.062
year + chemistry	_	_	_	_	_	0.013	_	_	-0.005	_	_	0.024	_	_	0.003	_	_	0.008	_	_
residuals	_	0.933	_	_	_	0.926	_	_	0.959	_	_	0.904	_	_	0.940	_	_	0.972	_	_
year (speciesxchemistry)	1	0.004	1.531	**0.001**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
species	4	0.028	1.913	**0.001**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
species (yearxchemistry)	4	0.018	1.563	**0.001**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
chemistry(yearxspecies)	5	0.030	1.787	**0.001**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Supplementary data 3 Results of PERMANOVA tests and correlations between wood properties and the NMDS analyses, based on Jaccard dissimilarity matrix built from the OTU presence-absence table, for the whole model considering all samples and for each tree species independently. Values in bold are significant at $P < 0.05$.

	Whole model					***Acer campestre***					***Carpinus betulus***					***Fraxinus angustifolia***					***Quercus robur***					***Ulmus laevis***				
Adonis F-statistics from PERMANOVA tests																														
	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p	Df	Sum of squares	R^2	F	p
Age class	1	1.11	0.02	2.75	**0.001**	1	0.52	0.05	1.30	**0.027**	1	0.48	0.05	1.17	0.115	1	0.69	0.07	1.75	**0.002**	1	0.56	0.06	1.46	**0.016**	1	0.53	0.05	1.26	**0.010**
Tree species	4	2.89	0.05	1.79	**0.001**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Age class x Tree species	4	1.93	0.04	1.20	**0.005**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Residual	117	47.19	0.89			24	9.72	0.95			23	9.44	0.95			24	9.46	0.93			24	9.31	0.94			22	9.26	0.95		
Total	126	53.11	1.00			25	10.24	1.00			24	9.92	1.00			25	10.15	1.00			25	9.87	1.00			23	9.79	1.00		
Homogeneity of dispersion between PERMANOVA groups																														
	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p	Df	Sum of squares	Mean square	F	p
Groups	18	0.16	0.009	5.106	**<0.001**	3	0.04	0.014	10.55	**<0.001**	2	0.01	0.003	1.12	0.345	3	0.01	0.005	5.20	**0.007**	3	0.04	0.012	4.69	**0.011**	3	0.04	0.012	12.50	**<0.001**
Residuals	108	0.19	0.002			22	0.03	0.001			22	0.06	0.003			22	0.02	0.001			22	0.06	0.003			20	0.02	0.001		
Correlations between wood properties and the NMDS analyses																														
	r2	p				r2	p				r2	p				r2	p				r2	p				r2	p			
age	0.33	**0.001**				0.28	**0.018**				0.20	0.075				0.60	**0.001**				0.29	**0.017**				0.35	**0.011**			
water	0.31	**0.001**				0.45	**0.004**				0.58	**0.001**				0.78	**0.001**				0.43	**0.002**				0.18	0.116			
N	0.04	0.061				0.14	0.165				0.09	0.342				0.13	0.197				0.18	0.109				0.13	0.231			
C	0.02	0.341				0.06	0.482				0.09	0.384				0.01	0.886				0.04	0.625				0.24	0.057			
C/N	0.06	**0.014**				0.21	0.066				0.14	0.204				0.17	0.118				0.13	0.210				0.07	0.416			
pH	0.60	**0.001**				0.66	**0.001**				0.10	0.331				0.57	**0.001**				0.58	**0.001**				0.48	**0.002**			
ergosterol	0.19	**0.001**				0.34	**0.004**				0.38	**0.003**				0.64	**0.001**				0.55	**0.001**				0.07	0.483			

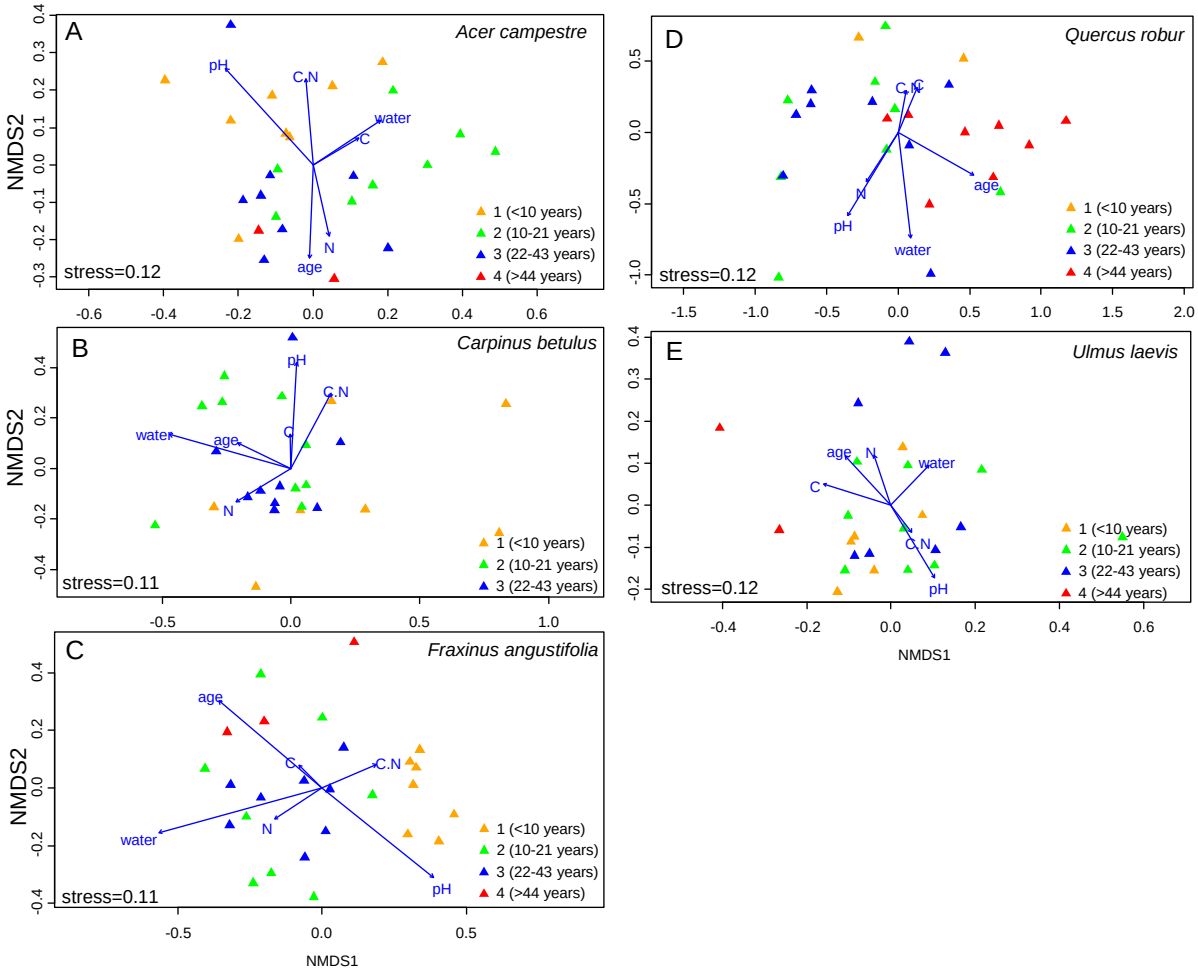
Supplementary data 4 Successional change in fungal community composition of the natural floodplain forest. The plots represent the correlations of Jaccard dissimilarity of dead wood fungal communities (built from the OTU presence-absence table) and dead wood age class distances. Mantel correlation tests indicate the significance of the correlation between the Jaccard dissimilarity matrix and the age matrix.



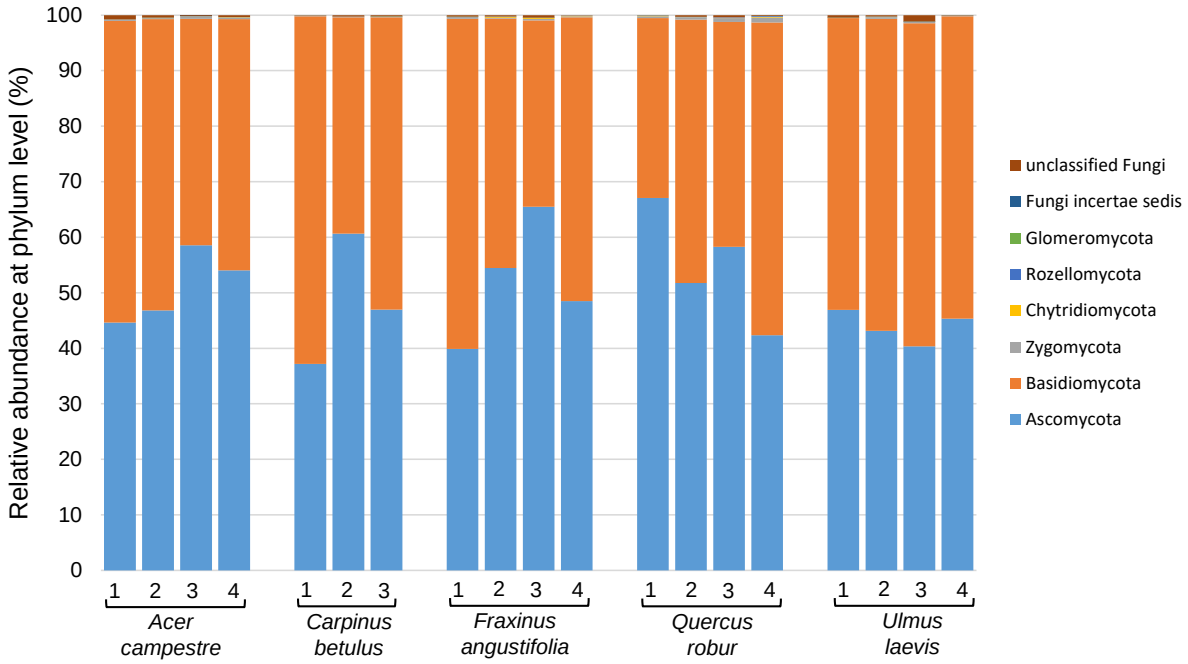
Supplementary data 5 Diversity of fungal communities in decomposing dead wood in the natural floodplain forest. Values represent means ± standard errors. Diversity calculations were performed after subsampling to 10,000 sequences.

	Age class	Decomposition time	n	Shannon	OTU Richness	Chao1
Acer campestre						
	1	<10 years	8	2.96 ± 0.23	448 ± 51	922 ± 87
	2	10-21 years	7	3.05 ± 0.16	498 ± 54	1116 ± 115
	3	22-43 years	8	2.99 ± 0.17	585 ± 48	1373 ± 154
	4	>44 years	2	2.88 ± 0.54	616 ± 214	1271 ± 338
Carpinus betulus						
	1	<10 years	6	2.11 ± 0.33	366 ± 75	822 ± 178
	2	10-21 years	9	2.83 ± 0.23	436 ± 58	983 ± 122
	3	22-43 years	9	2.9 ± 0.21	517 ± 60	1091 ± 121
	4	>44 years	0	_	_	_
Fraxinus angustifolia						
	1	<10 years	7	3.26 ± 0.47	547 ± 112	1091 ± 193
	2	10-21 years	8	3.18 ± 0.24	512 ± 79	1293 ± 201
	3	22-43 years	8	3.15 ± 0.29	540 ± 61	1429 ± 205
	4	>44 years	3	3.65 ± 0.58	759 ± 380	1776 ± 806
Quercus robur						
	1	<10 years	2	3.99 ± 0.11	643 ± 103	1177 ± 207
	2	10-21 years	7	3.49 ± 0.37	581 ± 83	1148 ± 128
	3	22-43 years	8	3.49 ± 0.43	657 ± 108	1377 ± 266
	4	>44 years	7	3.4 ± 0.21	513 ± 62	941 ± 104
Ulmus laevis						
	1	<10 years	5	3.14 ± 0.53	565 ± 105	1240 ± 246
	2	10-21 years	7	2.66 ± 0.56	503 ± 101	1123 ± 204
	3	22-43 years	6	2.49 ± 0.27	351 ± 33	840 ± 81
	4	>44 years	2	2.83 ± 0.53	391 ± 97	713 ± 221

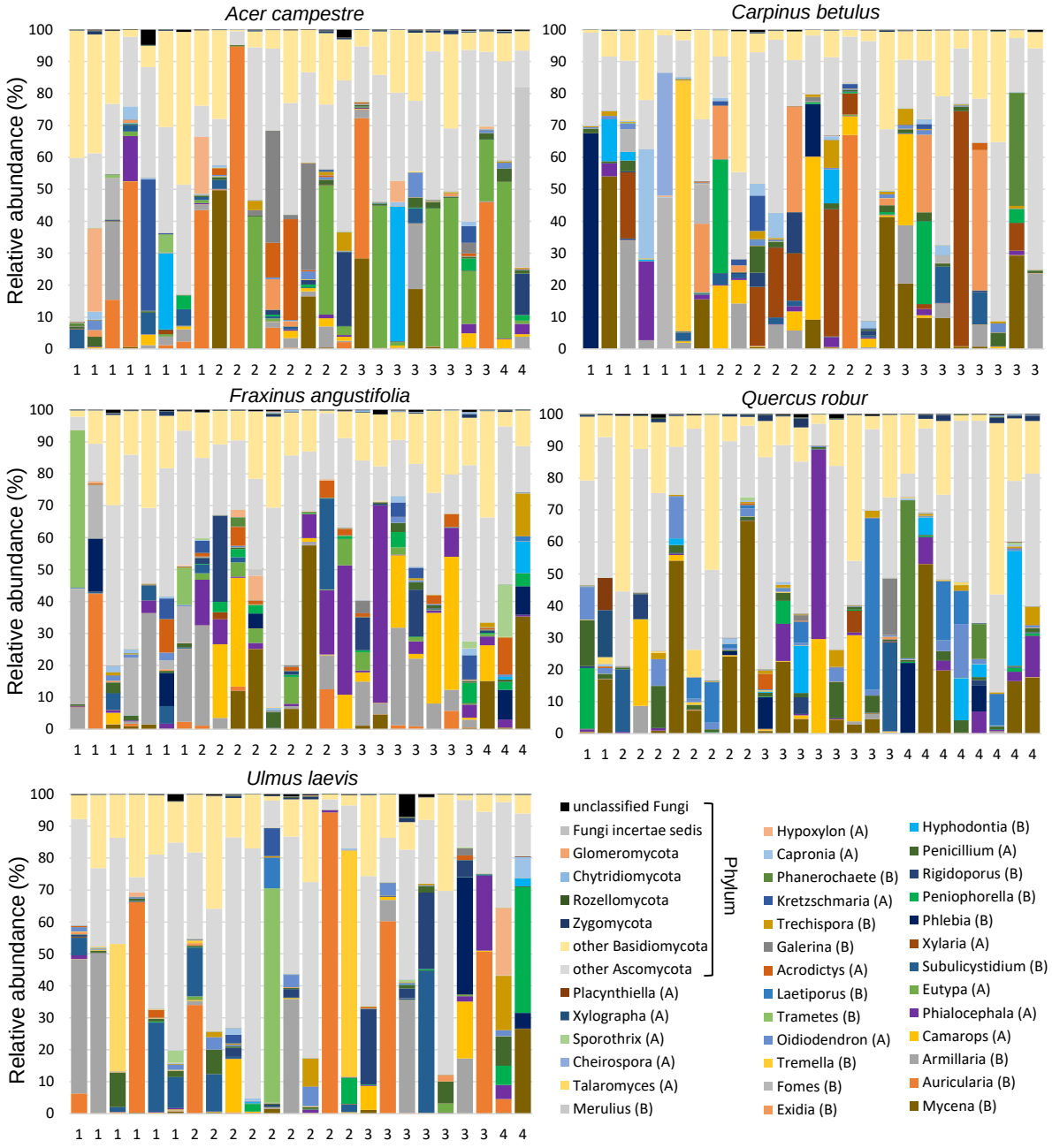
Supplementary data 6 Nonmetric multidimensional scaling (NMDS) of fungal communities in the dead wood samples from the natural floodplain forest separated by tree species. The NMDS analysis is based on the Bray-Curtis dissimilarities; vectors indicate environmental variables showing a significant effect.



Supplementary data 7 Taxonomic placement of fungi occurring in decomposing dead wood in the natural floodplain forest. Numbers indicate the age class of the dead wood.



Supplementary data 8 Fungi occurring in decomposing dead wood in the natural floodplain forest. The data represent means of relative abundances of fungal genera in individual CWDs. Abbreviations: A: Ascomycota, B: Basidiomycota. Numbers indicate the age class of the dead wood. Only genera with at least 5% relative abundance in one of the treatments are specified, and all others are classified to the phylum rank.



Supplementary data 9 List of the abundant fungal taxa in the natural floodplain forest with their properties. 220 OTUs with relative abundance >0.5% in at least three CWDs, their tree specificity and placement in succession are shown as well as the number of CWDs where the taxon occurs. Abbreviations: AC: *Acer campestre*, CB: *Carpinus betulus*, FA: *Fraxinus angustifolia*, QR: *Quercus robur*, UL: *Ulmus laevis*.